Published in final edited form as:

J Cardiovasc Transl Res. 2017 June ; 10(3): 285–294. doi:10.1007/s12265-017-9729-1.

Phenotype-Specific Association of Single-Nucleotide Polymorphisms with Heart Failure and Preserved Ejection Fraction: a Genome-Wide Association Analysis of the Cardiovascular Health Study

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Abstract

Little is known about genetics of heart failure with preserved ejection fraction (HFpEF) in part because of the many comorbidities in this population. To identify singlenucleotide polymorphisms (SNPs) associated with HFpEF, we analyzed phenotypic and genotypic data from the Cardiovascular Health Study, which profiled patients using a 50,000 SNP array. Results were explored using novel SNP- and gene-centric tools. We performed analyses to determine whether some SNPs were relevant only in certain phenotypes. Among 3804 patients, 7 clinical factors and 9 SNPs were significantly associated with HFpEF; the most notable of which was rs6996224, a SNP associated with transforming growth factor-beta receptor 3. Most SNPs were associated with HFpEF only in the absence of a clinical predictor. Significant SNPs represented genes involved in myocyte proliferation, transforming growth factor-beta/erbB signaling, and extracellular matrix formation. These findings suggest that genetic factors may be more important in some phenotypes than others.

Keywords

Heart failure with preserved ejection fraction; Genome-wide association study; Heart failure phenotype; Comorbidities; Atrial fibrillation; Chronic obstructive pulmonary disease; Coronary artery disease; Hypertension; Chronic kidney disease

Introduction

Approximately half of all heart failure patients have a preserved ejection fraction (HFpEF). Unlike dilated cardiomyopathies, little is known about potential genetic determinants of HFpEF. HFpEF patients are a heterogenous population with multiple comorbidities that likely contribute both to the pathogenesis of heart failure and patient symptom burden [1, 2].

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Associate Editor Sanjiv Shah oversaw the review of this article

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Consequently, it may be difficult to distinguish genetic determinants of HFpEF from the influence of comorbidities, because certain genetic factors may be important only in the absence or presence of certain pathophysiologic processes. It is therefore possible that genetic determinants of HFpEF vary between different clinical backgrounds.

To date, no intervention has been shown to improve clinical outcomes in the HFpEF population as a whole [3]. Given the heterogeneity of the HFpEF population, it has been proposed that targeting complex, specific HFpEF phenotypes may reveal effective therapeutic options based on phenotype-specific pathophysiology [1, 4]. Furthermore, complex HFpEF phenotypes may have significant prognostic implications beyond what is predicted by simple phenotype components, and such phenotypes may be associated with differential treatment response [5, 6]. Identification of genetic HFpEF determinants may also be phenotype specific, and these relationships may provide deeper insight into the mechanism of HFpEF within these phenotypes and possible therapeutic interventions.

The availability of large, longitudinal epidemiologic studies with accompanying genomic data provides the opportunity to identify associations between complex patient phenotypes and HFpEF. Herein, we present an analysis of the genomic associations between HFpEF and patient phenotypes in the Cardiovascular Health Study (CHS) [7].

Methods

This study was approved by the Colorado Multiple Institutional Review Board. Clinical and genomic data from the CHS (phs000287.v5) [7] for all subjects were obtained from the Database of Genotypes and Phenotypes (National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD) [8].

Study Population

The CHS is a large, longitudinal population-based study designed to study cardiovascular disease including risk factors and outcomes [7]. It enrolled 5888 subjects ≥65 years old from 4 geographically diverse locations between 1991 and 2009. Patients underwent historical (including symptoms, psychosocial evaluation, medical history, and medications), physical, laboratory, and imaging evaluation at baseline and at annual follow-up. Evaluations included echocardiography at baseline (original cohort) at 2 years (supplemental cohort) and at 7 years [9]. Major outcomes of interest were all-cause mortality, myocardial infarction, angina, congestive heart failure, stroke, and transient ischemia attack [10].

Study Adjudication of Heart Failure Events

CHS study protocols for identifying HF both at baseline and in follow-up have been described previously [11]. Briefly, CHS criteria required that a patient (a) have a diagnosis of HF from a physician and (b) be under medical treatment for HF, defined as being treated with a diuretic and a vasodilator (e.g., angiotensin-converting enzyme inhibitor, nitrate, or hydralazine). Physician-reported HF events were verified using subject medical records. HF events were adjudicated individually by an expert panel that reviewed clinical documentation (inpatient and outpatient) including history, physical examination, radiography, and medications.

Classification of HFpEF

In the present analysis, HFpEF was defined as the presence of clinical HF and a documented EF recorded as either normal, borderline, or mildly reduced. Subjects who either had HF at baseline or developed it within the first 7 years and had a left ventricular ejection fraction (LVEF) value at both baseline and 7 years showing normal or mildly reduced EF were classified as HFpEF. Patients with an echocardiogram showing moderately or severely reduced EF at baseline or 7 years were excluded, as were patients with documented HF without an EF result. All subjects who developed HF more than 7 years after enrollment were excluded due to the lack of EF data beyond year 7. Subjects who did not develop HF at any point during the study were included as controls. The primary analysis used presence of HFpEF at any point vs. remaining subjects without HFpEF. Secondary analyses compared patients with controls with baseline and incident HFpEF subjects separately given the lower burden of comorbidities in subjects who developed HFpEF at a younger age.

Patient Phenotyping

Clinical covariates were selected based on prior studies describing an association with HFpEF [1, 12]. Subjects were analyzed according to enrollment age; sex; race; body mass index (BMI); and baseline presence of hypertension (HTN), diabetes mellitus (DM), atrial fibrillation (AF), coronary artery disease (CAD), anemia (hemoglobin <13 g/dL in men, hemoglobin <12 g/dL in women), chronic obstructive pulmonary disease (COPD), or chronic kidney disease (CKD) ≥ stage 4 defined as creatinine clearance <30 mL/min/1.73 m^2 using the Modified Diet in Renal Disease equation [13].

Echocardiographic variables were present with varying degrees of completeness at baseline and at 7-year follow-up. Indices relevant to HFpEF including LV wall thickness, calculated LV mass, left atrial size, mitral inflow *E* wave, *A* wave, and EA ration as well as circumferential LVend-systolic stress as calculated by the CHS core lab were summarized for baseline, incident, and all HFpEF patients as well as controls at both time points.

Genome-Wide Analysis Quality Control

Subjects who provided informed consent for participation in genomic analysis were genotyped using the ~50,000 singlenucleotide polymorphism (SNP) ITMAT-Broad-CARe (IBC) genotyping version two array (Illumina, San Diego, CA, USA) [14]. The IBC array was constructed using genetic loci and SNPs implicated in vascular disease including coronary artery disease, metabolic pathways, and inflammation based on an extensive literature search in 2008. Of note, studies of HF were not included in construction of this array. SNPs with a call rate <95% or a minor allele frequency of <1% in the CHS population were excluded. SNPs that violated the Hardy-Weinberg equilibrium ($p < 10^{-6}$) in control patients were also removed. Subjects with discordance between reported sex and observed *X* and *Y* chromosomes, with a sample call rate <95% and with an inbreeding coefficient >0.10, were excluded. After applying linkage disequilibrium pruning (cutoff 0.2), potentially related subjects were identified based on an identity by kinship coefficient >0.1, and the individual with the lower genotype call rate of related pairs was removed. Primary ancestry groups were identified using principal component analysis. There were three primary clusters according to the first two principal components; two of which were similar and

comprised nearly exclusively of self-reported white subjects, whereas the third represented the African American subjects. For the present analysis, both clusters of white patients were included. Reported minor allele frequencies represent those observed in the CHS study using the IBC array.

Statistical Analysis

Significant associations between clinical covariates and HFpEF were identified using step-forward multivariate logistic regression using $p < 0.05$ as an entry threshold. Patients with data available for all multivariate predictors of HFpEF were also classified according to presence or absence of at least one multivariate risk factor (≥1 risk factor present vs. none).

Associations between HFpEF, multivariate clinical predictors, and SNPs were evaluated by logistic regression using dominant, additive, and recessive models for the minor allele. Associations between HFpEF and SNPs with and without multivariate clinical predictors were characterized in a similar fashion. Using the Bonferroni correction, an association p value $<5 \times 10^{-7}$ was considered significant and $<1 \times 10^{-6}$ was considered suggestive of association in genome-wide analysis. All genome-wide analyses were performed using PLINK [15, 16]. All other analyses were performed using the R statistical package (version 3.3.0; R Core Team, Vienna, Austria).

Knowledge-Based SNP Analysis

We created a computational tool incorporating dbSNP [17], Hapmap [18], and PharmGKB [19] into a SNP-centric network that can be used for knowledge-based analysis and interpretation of genome-wide association analysis results. In these networks, nodes represent SNPs and edges represent their relationships extracted from the knowledge bases listed above. The three integrated resources facilitated identification of genes, location, mutation type, and inheritance patterns (e.g., D', R^2, and logarithm of odds values for linkage disequilibrium) associated with SNPs of interest. We used RenoDOI [20, 21], a knowledge-based visual analysis platform for Cytoscape (version 3.2.1) [22], to perform both SNP- and gene-centric network analyses in order to explore relationships between SNPs, genes, and phenotypes to identify possible mechanisms. Knowledge sources for gene-based analysis have been described previously [20, 21].

Results

After quality control procedures, 36,189 SNPs and 3804 subjects were included. Of these subjects, 80 (2.1%) had a reduced ejection fraction at one of the time points and 686 (18.0%) had HF of unknown type resulting in exclusion from subsequent analysis. This left a total of 3038 subjects including 284 (9.3%) HFpEF cases and 2754 (90.7%) controls. Cases included 123 (4.5%) with HFpEF at baseline and 161 (5.8%) who developed HFpEF within the first 7 years of enrollment. Baseline characteristics of control and HFpEF patients are summarized in Table 1. All clinical factors were significantly different in all HFpEF subjects vs. controls as expected based on prior observations [1, 12, 23]. Subjects with HFpEF at baseline were older and more likely to have AF, HTN, COPD, CAD, DM, anemia, and CKD compared to controls. Subjects with incident HFpEF during enrollment were more likely at

baseline to be older and male with a higher BMI, AF, HTN, COPD, CAD, or DM compared to controls.

The overrepresentation of men in HFpEF cases was likely due to the inclusion of borderline or mildly abnormal ejection fraction in HFpEF cases. For example, women were more likely overall to have a normal baseline LVEF (2071/2210, 95.0%) compared with men (1365/1594, 86.4%, $p < 0.001$). However, 21/123 (17.1%) baseline HFpEF cases had a borderline or mildly abnormal LVEF compared with 115/2754 (4.2%) controls ($p < 0.001$). When stratified by LVEF, there were no significant sex differences between prevalence of in subjects with normal baseline LVEF ($p = 0.12$) or borderline/mildly abnormal LVEF ($p = 0.13$).

Multivariate odds ratios (ORs) for all, baseline, and incident HFpEF cases are shown in Fig. 1. Baseline age, CAD, AF, and DM were significantly associated with all HFpEF cases. HTN was significantly associated with all and incident HFpEF. Anemia was significantly associated with all and baseline HFpEF, and CKD was associated with baseline HFpEF only. Available echocardiographic measurements are summarized in Table 2. As expected, subjects with HFpEF had significantly greater posterior LV wall thickness, LV mass, atrial size, and LVend-systolic stress at both time points compared with controls. In addition, subjects with HFpEF were more likely to have abnormal E/A ratio (either <1 or >1.5) at baseline.

SNPs and related genes associated with HFpEF are shown in Table 3. Among all 3038 subjects, 1 SNP was significantly associated with HFpEF. The rs6696224 (minor allele frequency 11.2%) was associated with all and incident HFpEF (OR 7.6 and 11.3, respectively). In total 7/36,189 (0.02%) SNPs were at least possibly associated with at least 1 of the HFpEF groups ($p < 1 \times 10^{-6}$). The majority of associations were found using a recessive model (10/12 significant, 4/7 suggestive associations).

In total, 728/2935 (24.8%) subjects with no missing data had no multivariate clinical predictors of HFpEF. Among these patients, rs2466052 (OR 10.1) and rs10759715 (OR 4.6) were significantly associated with HFpEF. In comparison, rs5871 (OR 11.7) was significantly associated with HFpEF in patients with at least one risk factor. The rs6696224 remained significantly associated with incident HFpEF (OR 9.5) and possibly associated with all HFpEF cases (OR 8.5) when adjusted for the number of risk factors present. When subdividing subjects into groups with and without specific multivariate clinical risk factors for HFpEF, there were 15 associations comprised of 5 unique SNPs. The following two SNPs were significantly associated with HFpEF in the absence of specific risk factors: rs6696224 (AF, CAD, COPD, HTN) and rs3823879 (DM).

The following three SNPs had a suggestive association with HFpEF in the absence of specific risk factors: rs604983 (CKD), rs2762941 (COPD), rs882520 (DM). There were four significant associations with all HFpEF cases, one with baseline, and seven with incident HFpEF. Of these associations, the strongest was between rs6696224 and incident HFpEF in the absence of HTN (OR 25.3, $p = 3.6 \times 10^{-10}$). Five suggestive associations were with all HFpEF cases, one was with baseline, and one was with incident HFpEF. Analyzing the

SNP- and gene-centric knowledge networks, we noted that SNPs were distributed over a number of chromosomes (1, 7, 8, 9, 19, 20, max = 2/chromosome). Of the seven HFpEF-associated SNPs (both significant and suggestive), six (85.7%) were intron variants, compared with 64.9% overall on the IBC array [14]. The only non-intron SNP was rs5871 (3′ untranslated region).

SNPs significantly associated with HFpEF were related to TGFBR3 (rs6696224), NRG1 (rs2466052), and ELN (rs3823879). Suggestive SNPs were related to CALM1 (rs5871), BCL9 (rs604983), CYP24A1 (rs2762941), and ARHGEF1 (rs882520). When considering significant genes only, annotation cluster analysis using DAVID [24] revealed overrepresentation of Gene Ontology (GO) annotations of skeletal muscle development and cell proliferation (TGFBR3, ELN, NRG1) as well as for cellular signaling transduction (TGFBR3, NRG1) [25]. When considering all genes possibly associated with HFpEF, the most significant GO annotation clusters were transmembrane receptor signaling including cell proliferation and muscle development (TGFBR3, ELN, NRG1, ARHGEF1) and cell cycle regulation (TGFBR3, NRG1, CALM1, BCL9). Represented genes were associated with Kyoto Encyclopedia of Genes and Genomes pathways involved in extracellular matrix regulation (TGFBR3, ELN), vascular smooth muscle contraction (CALM1, ARHGEF1), and ErbB signaling (NRG1) [26].

Discussion

Among Caucasian subjects from the CHS, seven clinical factors and three SNPs (rs6696224, rs2466052, and rs3823879) were significantly associated with HFpEF either at the time of enrollment or which developed during the first seven years. These SNPs, most notably rs6696224 which is associated with TGFBR3, are among the first to be implicated in clinical HFpEF. Our findings redemonstrate that HFpEF is strongly associated with a number of clinical comorbidities and suggest that genetic risk factors may be most relevant in certain phenotype backgrounds. The majority of phenotype-specific significant SNPs were observed in the absence of comorbidities such as AF, CAD, HTN, COPD, and DM. The significance of this observation is unclear but could reflect that genetic factors are more important for development of HFpEF in the absence of certain known pathologic processes that contribute to HFpEF development [1]. For example, TGFBR3 is a component of the transforming growth factor-beta signaling pathway, which has been implicated in several pathologic processes associated with HFpEF, indicating the rs6696224 is biologically plausible as a contributor to HFpEF pathogenesis. Conversely, when comorbidities are present, related pathologic processes might be more important than genetic factors in HFpEF development. Further study of possible interactions between these genetic variants, specific comorbidities, and HFpEF may provide additional insight into different mechanisms of HFpEF development.

The rs6696224, an intron variant of TGFBR3, was significantly associated within the entire study population as well as in several specific phenotype groups (e.g., normotensive, non-diabetic). TGFBR3 encodes a TGF-β receptor 3, transmembrane proteoglycan, which has been implicated in both myocardial hypertrophy and fibrosis [27, 28]. TGF-β3 appears to decrease with pressure overload and immediately following myocardial infarct followed by a

prolonged increased level a rat model compared with sham control animals [29]. Overexpression of TGF-β3 has also been associated with decreased cardiac fibrosis, possibly due to inhibition of TGF-β1/2 signaling [30]. The relationships between TGF-β3, cardiomyocyte hypertrophy, and cardiac fibrosis suggest that variation in the TGF-β signaling pathway could plausibly affect development of left ventricular hypertrophy and diastolic function; both of which are commonly associated with HFpEF. Furthermore, TGFBR3 has been suggested as a possible therapeutic target to treat cardiac fibrosis [30–32]. The functional significance rs6696224 is unknown, although the rs3917187 polymorphism, an intron variant in the TGFB3 gene encoding TGF-β3, has been associated with variation in LV mass and ventricular dimensions [33].

In addition to TGF-β3, elastin (encoded by ELN) has been observed to be implicated in LV hypertrophy and heart failure. Specifically, decreased elastin/collagen ratio has been associated with worsening ventricular stiffness [34], and elastin overexpression limits scar expansion and preserves LV function following MI [35]. Given that TGF-β3 blocks collagen TGF-β1/2-mediated collagen production, it is plausible that these two gene products may be involved in HFpEF pathogenesis.

Neuregulin-1, encoded by NRG1, has also been associated with cardiac performance and hypertrophy via activation of ErbB2/4 receptors by an epidermal growth factor-like domain [36]. NRG1 expression has been observed to increase during the development of concentric LV hypertrophy with sharp decreases once LV dilation starts [37]. NRG1 expression has been shown to induce expression of atrial natriuretic factor and initiates organization of myofibrillar cardiomyocyte bundles, thereby attenuating myocardial hypertrophy and protecting the heart from pathologic remodeling resulting in dilated cardiomyopathies [38]. However, the role of neuregulin-1 in development of HFpEF remains unclear.

Myocardial structure, function, and tissue-level characterizations in CHS were insufficient to explore possible mechanisms between HFpEF, comorbidities, and SNPs directly. However, existing evidence from animal models supports the possibility that genetic variants are more important in the absence of a comorbid condition. For example, it has been demonstrated that in DM, TGF-β is induced and activated in multiple organ systems contributing to cardiac, renal, pulmonary, and cutaneous fibrosis via Smad3-mediated activity [39]. TGFBR3 and ELN were both significantly associated with HFpEF in the absence of DM. This observation might be because the effect of decreases in TGFBR3 activity, which would promote fibrosis due to decreased inhibition of the TGF-β3/Smad2/3 pathway [30], might be insignificant in the setting of DM by the strong activation of the TGF-β pathway. Similarly, decreases in elastin bioactivity due to genetic factors may be relatively unimportant in light of the excess myocardial collagen deposition and consequent unfavorable shift of myocardial elastin/collagen ratio that results from TGF-β/Smad 3 activation [40]. These speculative mechanisms cannot be evaluated directly in this study but may explain the observation that these two genes were associated with HFpEF only in the absence of DM.

In this study, we examined only SNPs associated with HFpEF in the presence and absence of clinical predictors of HFpEF in the CHS population (Fig. 2), but there are many potential interactions between individual phenotypes and genotypes as determinants of HFpEF.

Comorbidities associated with HFpEF may contribute to progression of HFpEF through a number of pathways, which may be specific to each comorbidity [41–43]. These putative mechanisms include multiple signaling pathways such as renin-angiotensin-aldosterone signaling, TGF-β-mediated fibrosis, protein kinase G activity, nitric oxide bioavailability, and inflammatory pathways [41]. SNP-HFpEF associations in the absence of a phenotype such as HTN may represent pathways important to HFpEF pathophysiology that are related to the physiology of that phenotype. When the phenotype is present, genetic alterations in that pathway might be less important, whereas when the phenotype is absent, genetic alterations in a given pathway may have more pathophysiologic significance. It is also possible that other SNPs not included in this analysis may be particularly relevant both in the presence and absence of key HFpEF-associated comorbidities. Although the IBC array was designed to focus on SNPs associated with cardiovascular disease, it focused on blood pressure, insulin resistance, metabolic disease, and inflammation but not specifically on heart failure or cardiomyopathies [14]. Consequently, SNPs associated with sarcomeric function were not well represented and may also have phenotype-specific associations with HFpEF. Likewise, other unanticipated, novel pathways may be involved in HFpEF development both with and without specific phenotypes. These questions will be the subject of future work using more expansive SNP arrays.

Limitations

Performing phenotype-specific association analysis increases the potential for false discovery through additional tests and due to small numbers of cases. Unfortunately, validation in other similar dbGaP datasets was not possible due to publication embargo policies. Similarly, the overrepresentation of recessive risk models among significant and suggestive SNP-HFpEF associations may also be a result of the smaller patient populations, although there were SNPs associated with HFpEF using dominant or additive models, where at-risk genotypes represented a majority of the population. Most of the identified genes were intron variants, and there is limited knowledge regarding the associations between intron variants and either protein expression or function. Still, intron variants may have functional effects on gene activity that have yet to be determined. Additional insight might be gained using genotype imputation to identify associated exon variants, but the sparse coverage of the IBC SNP array limits the quality of imputation in the CHS. As stated earlier, IBC array was based on involvement in vascular diseases, certain metabolic pathways, and inflammatory processes based on data available prior to 2008. This prevented evaluation of phenotypespecific SNP associations outside of these categories and of SNPs recently discovered to be associated with the disease domains of interest. SNP-HFpEF associations should be validated and possibly expanded in independent patient populations. For consistent observations in multiple populations, mechanisms should be explored where possible using animal or cell-line models.

Conclusion

The SNP rs6696224, an intron variant of TGFBR3, was significantly associated with HFpEF in a large population of elderly Caucasian patients. Additional SNPs were associated with HFpEF in certain phenotype contexts, generally the absence of known clinical predictors of HFpEF. Further work is needed to validate these observations and expand knowledge

regarding genetic determinants of HFpEF including the mechanisms of their phenotypic dependence and opportunities for targeted therapy.

Acknowledgments

The authors would like to thank William Baumgartner for his role in creating the gene-centric knowledge networks for this analysis.

Sources of Funding This work was supported the American Heart Association Cardiovascular Phenome-Genome Study Award 15CVGPSD27090024.

Abbreviations

AF	Atrial fibrillation
BMI	Body mass index
CAD	Coronary artery disease
CHS	Cardiovascular Health Study
CKD	Chronic kidney disease
COPD	Chronic obstructive pulmonary disease
DM	Diabetes mellitus
GWA	Genome-wide association
HFpEF	Heart failure with preserved ejection fraction
IBC	ITMAT-Broad-CARe
HTN	Hypertension
OR	Odds ratio
SNP	Single-nucleotide polymorphism
TGF	Transforming growth factor

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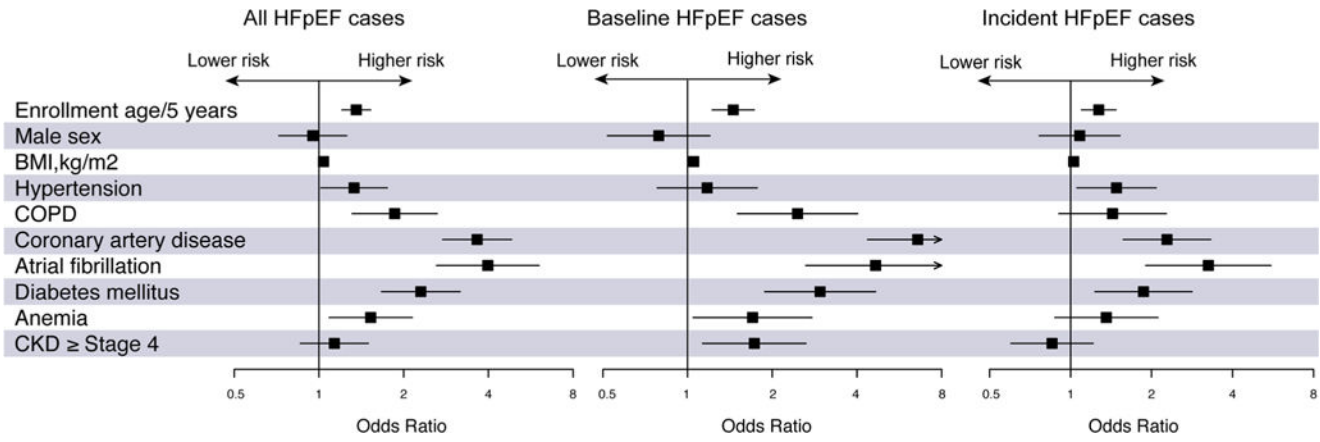

Fig. 1.
Multivariate predictors of HFpEF groups

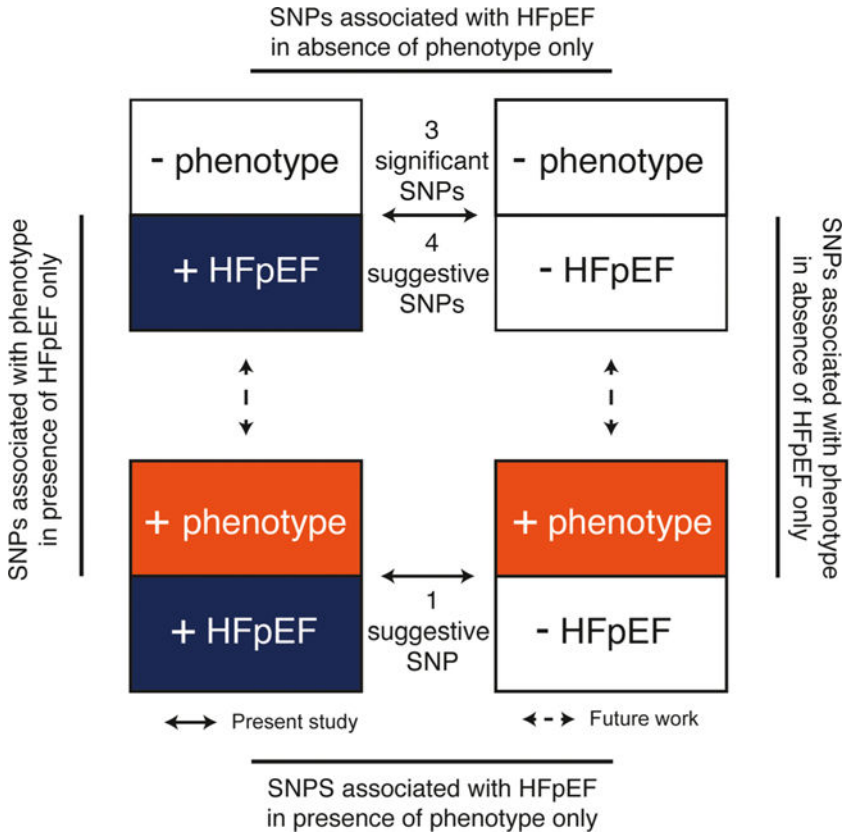

Fig. 2.

Schematic of potential interactions between genetic and clinical determinants of HFpEF

Table 1

Baseline characteristics according to HFpEF status

	No HFpEF	Baseline HFpEF	Incident HFpEF	Any HFpEF
Characteristics	2754 (90.7)	123 (4.5)	161 (5.8)	284 (9.3)
Age, years	71 (68–75)	76 (71–79) ***	74 (70–77) ***	74 (70–78) ***
Male, *N*(%)	1084 (39)	56 (46)	77 (48) *	133 (47) *
Atrial fibrillation, *N*(%)	101 (4)	26 (21) ***	21 (13) ***	47 (17) ***
Hypertension, *N*(%)	1049 (38)	67 (54) ***	86 (53) ***	153 (54) ***
COPD, *N*(%)	316 (12)	29 (24) ***	25 (16)	54 (20) ***
Coronary artery disease, *N*(%)	551 (20)	73 (59) ***	66 (41) ***	157 (55) ***
BMI, kg/m^2—median (Q1–Q3)	25.7 (23.2–28.5)	24.9 (23.1–29.9)	26.3 (24.1–29.0) *	26.1 (23.5–29.4) *
Diabetes mellitus, *N*(%)	316 (12)	38 (31) ***	36 (22) ***	74 (26) ***
Anemia, *N*(%)	378 (14)	30 (25) ***	29 (18)	59 (21) ***
≥Stage 4 CKD, *N*(%)	1104 (40)	79 (64) ***	68 (42)	147 (52) ***

COPD chronic obstructive pulmonary disease, *BMI* body mass index, *CKD* chronic kidney disease ≥stage 4

Q1 quartile 1, *Q3* quartile 3

* $p < 0.05$,

** $p < 0.01$,

*** $p < 0.001$ vs. control (no HFpEF)

Table 2

Echocardiographic characteristics according to HFpEF status

Characteristics	No HFpEF *N* (%) 2754 (90.7)	Baseline HFpEF *N* (%) 123 (4.5)	Incident HFpEF *N* (%) 161 (5.8)	Any HFpEF *N* (%) 284 (9.3)
Baseline echocardiogram—median (Q1–Q3), *N (%)* values reported				
Posterior LV wall thickness, diastole (cm)	0.82 (0.74–0.91)	0.86 (0.78–0.96) **	0.87 (0.80–0.98) ***	0.87 (0.79–0.97) ***
	1839 (67)	*73 (59)*	*113 (70)*	*186 (65)*
Calculated LV mass (g)	135 (112–164)	174 (129–210) ***	156 (130–200) ***	160 (129–205) ***
	1839 (75)	*73 (59)*	*113 (70)*	*186 (65)*
LV mass/body surface area (g/m^2)	79 (67–93)	98 (73–122) ***	90 (78–111) ***	90 (75–115) ***
	1836 (67)	*73 (59)*	*112 (70)*	*185 (65)*
Left atrial dimension, *M* mode (cm)	3.8 (3.4–4.2)	4.4 (3.8–5.1) ***	4.1 (3.7–4.6) ***	4.2 (3.7–4.8) ***
	2657 (96)	*119 (97)*	*155 (96)*	*274 (96)*
Mitral inflow *E* wave velocity (cm/s)	69 (59–80)	77.5 (59–89) **	75 (61–91) ***	75 (61–90) ***
	2669 (97)	*116 (94)*	*155 (96)*	*271 (95)*
Mitral inflow *A* wave velocity (cm/s)	77 (65–90)	79 (52–94)	85 (65.5–101.5) **	83 (61–99)
	2668 (97)	*116 (94)*	*155 (96)*	*271 (95)*
E wave/*A* wave ratio, *N* (%)		***	**	***
1–1.5	764 (29)	27 (23)	32 (21)	59 (22)
<1	1781 (67)	65 (56)	109 (70)	174 (64)
>1.5	123 (5)	24 (21)	14 (9)	38 (14)
Circumferential LV ESS (g/cm^2)	57 (44–75)	57 (44–75) **	61 (43–78)	65 (44–81) **
	1826 (66)	*73 (59)*	*113 (70)*	*186 (65)*
Year 7 echocardiogram—median (Q1–Q3), *N (%)* values reported				
Posterior LV wall thickness, diastole (cm)	0.87 (0.79–0.96)	0.93 (0.80–0.96)	0.93 (0.93–1.04) ***	0.93 (0.92–1.03) ***
	1307 (47)	*30 (24)*	*110 (68)*	*140 (49)*
LV mass (g)	137 (112–167)	172 (138–220) ***	162 (136–205) ***	163 (137–211) ***
	1306 (47)	*30 (24)*	*110 (68)*	*140 (49)*
LV mass/body surface area (g/m^2)	77 (65–93)	98 (83–123) ***	92 (79–109) ***	92 (79–113) ***
	1293 (47)	*30 (24)*	*109 (68)*	*139 (49)*
Left atrial dimension, *M* mode (cm)	3.9 (3.5–4.3)	4.2 (3.7–4.9) **	4.3 (3.9–5.0) ***	4.3 (3.9–5.0) ***
	1800 (65)	*42 (34)*	*157 (98)*	*199 (70)*
Mitral inflow *E* wave velocity (cm/s)	69 (58–80)	76 (65–93) **	76 (63–92) ***	76 (64–92) ***
	1792 (65)	*39 (32)*	*148 (92)*	*187 (66)*
Mitral inflow *A* wave velocity (cm/s)	79 (66–92)	82 (76–91)	86 (71–107) ***	85 (71–102) ***
	1763 (64)	*35 (28)*	*132 (82)*	*167 (59)*
E wave/*A* wave ratio		***		**
1–1.5	459 (26)	8 (23)	26 (20)	34 (20)
≤1	1248 (71)	22 (63)	98 (74)	120 (72)
>1.5	53 (3)	5 (14)	8 (6)	13 (8)

Characteristics	**No HFpEF** ***N*** **(%)** **2754 (90.7)**	**Baseline HFpEF** ***N*** **(%)** **123 (4.5)**	**Incident HFpEF** ***N*** **(%)** **161 (5.8)**	**Any HFpEF** ***N*** **(%)** **284 (9.3)**
Circumferential LV ESS (g/cm^2)	53 (42–68)	68 (53–90) **	62 (46–80) **	63 (47–81) ***
	1202 (44)	*28 (23)*	*100 (62)*	*128 (45)*

Italics: Number of reported values (% of subjects with reported values)

Q1 quartile 1, *Q3* quartile 3, *LV* Left ventricle, *ESS* end-systolic stress

* $p < 0.05$,

** $p < 0.01$,

*** $p < 0.001$ vs. control (no HFpEF)

Table 3

SNPs associated with HFpEF by phenotype by genome-wide logistic regression

HFpEF	SNP	Gene	CHR	Position	MA	MAF	Model	GWA		At-risk genotype	
								OR	*p* value	Case	Control
All subjects											
All *	rs6696224	TGFBR3	1	91,966,250	G	0.112	REC	7.6	1.77E − 07	12/284 (4.2)	16/2754 (0.6)
Incident *	rs6696224	TGFBR3	1	91,966,250	G	0.112	REC	11.3	3.70E − 09	10/171 (6.2)	16/2754 (0.6)
No clinical risk factors											
All *	rs2466052	NRG1	8	32,631,182	G	0.049	ADD	10.1	2.76E − 07	8/23 (34.8)	35/701 (5.0)
All	rs10759715	–	9	116,317,116	G	0.188	ADD	4.6	9.39E − 07	15/23 (65.0)	143/705 (20.0)
Any risk factors present											
Baseline	rs5871	CALM1	14	89,941,231	G	0.092	REC	11.7	6.27E − 07	7/112 (6.2)	11/1943 (0.6)
Adjusted for number of risk factors present											
All	rs6696224	TGFBR3	1	91,966,250	G	0.112	REC	8.5	5.53E − 07	NA	NA
Incident *	rs6696224	TGFBR3	1	91,966,250	G	0.112	REC	9.5	2.4E − 07	NA	NA
No atrial fibrillation											
Incident *	rs6696224	TGFBR3	1	91,966,250	G	0.112	REC	11.3	1.24E − 08	9/140 (6.4)	16/2653 (0.6)
No coronary artery disease											
All	rs6696224	TGFBR3	1	91,966,250	G	0.112	REC	9.7	6.65E − 07	8/158 (5.1)	12/2379 (0.5)
Incident *	rs6696224	TGFBR3	1	91,966,250	G	0.112	REC	12.6	1.26E − 07	7/108 (6.5)	13/2379 (0.5)
No chronic kidney disease											
All	rs604983	BCL9	1	145,500,786	A	0.037	ADD	8.8	9.47E − 07	9/137 (6.6)	13/1637 (0.8)
No chronic obstructive pulmonary disease											
All *	rs6696224	TGFBR3	1	91,966,250	G	0.112	REC	8.9	9.89E − 08	11/220 (5.0)	14/2370 (0.6)
Incident *	rs6696224	TGFBR3	1	91,966,250	G	0.112	REC	14.0	4.94E − 10	10/130 (7.7)	14/2370 (0.6)
Incident	rs2762941	CYP24A1	20	52,217,059	A	0.400	REC	2.7	8.54E − 07	40/129 (31.0)	342/2370 (14.4)
No diabetes mellitus											
All	rs882520	ARHGEF1	19	47,081,120	T	0.489	ADD	1.7	8.47E − 07	161/207 (77.8)	1578 (65.7)
Baseline *	rs3823879	ELN	7	73,092,727	A	0.081	ADD	3.2	2.83E − 07	26/85 (30.6)	256/2425 (10.6)
Incident *	rs6696224	TGFBR3	1	91,966,250	G	0.112	REC	10.3	1.44E − 07	16/2425 (0.7)	8/125 (6.4%)

HFpEF	SNP	Gene	CHR	Position	MA	MAF	Model	GWA		At-risk genotype	
								OR	*p* value	Case	Control
No hypertension											
All *	rs6696224	TGFBR3	1	91,966,250	G	0.112	REC	17.5	3.22E − 09	10/131 (7.6)	8/1703 (0.5)
Incident *	rs6696224	TGFBR3	1	91,966,250	G	0.112	REC	25.3	3.61E − 10	8/75 (10.7)	8/1703 (0.5)

SNP single-nucleotide polymorphism, *CHR* chromosome, *MA(F)* minor allele (frequency), *OR* odds ratio, *GWA* genome-wide association, *CKD* chronic kidney disease, *COPD* chronic obstructive pulmonary disease, *ADD* additive, *REC* recessive

* $p < 5E-7$ (significant association using Bonferroni correction)